# Solving the Mystery of Anxiety

*'In view of the intimate connection between things physical and mental, we may look forward to a day when paths of knowledge will be opened up leading from organic biology and chemistry to the field of neurotic phenomena.'*
**— Sigmund Freud**

By PAMELA TAULBEE

When Freud wrote these words, he didn't know of neurotransmitters or receptors. Today, however, these are common terms in the lexicon of psychiatrists. Within the past few decades, these "paths of knowledge" through the brain's biochemical milieu have been traced by observing the effects of various drugs on biochemistry and behavior. Recently, scientists have shed new light on one aspect of behavior—anxiety—through the use of a class of drugs called benzodiazepines. These drugs, developed in the early 1970s, include the well-known Valium.

For years, even though no one knew exactly how they worked, benzodiazepines were used to treat patients with generalized anxiety. Now a whole body of new research suggests that benzodiazepines correct an imbalance in a chemical system in the brain that, scientists believe, exists to regulate anxiety.

"We are now at the stage with anxiety where we were with ... major psychiatric illnesses 20 or 30 years ago," says Phil Skolnick, a bio-organic chemistry researcher at the National Institute of Mental Health in Bethesda, Md. "Up until about five years ago, if you talked about the neurochemical basis of anxiety, people would laugh at you," he adds. These days, no one is laughing. Skolnick and his colleague Steven M. Paul have developed the first model of chemically induced anxiety in primates. This discovery, together with a vast amount of research at other mental health centers, has unmasked at least a portion of the mystery of anxiety.

In addition to synthesizing chemicals that induce or alleviate anxiety, several scientists have focused their research efforts on locating endogenous ligands — naturally occurring chemicals that work through the same brain cell mechanisms as benzodiazepines. Topping the list is the search for a natural Valium in the brain. However, some of the latest work has uncovered what appears to be a natural substance that *induces* anxiety. In either case, researchers believe the discovery of such a substance could revolutionize the treatment of anxiety.

In the clinical sense, anxiety encompasses feelings of apprehension and fear, which are accompanied by physiological changes such as increased blood pressure and heart rate, and a higher-than-normal blood level of stress hormones—cortisol, adrenocorticotropic hormone, and the catecholamines epinephrine and norepinephrine. This physiological and behavioral response is sometimes justified—the "flight-or-fight" response is, for the most part, physiologically identical to anxiety.

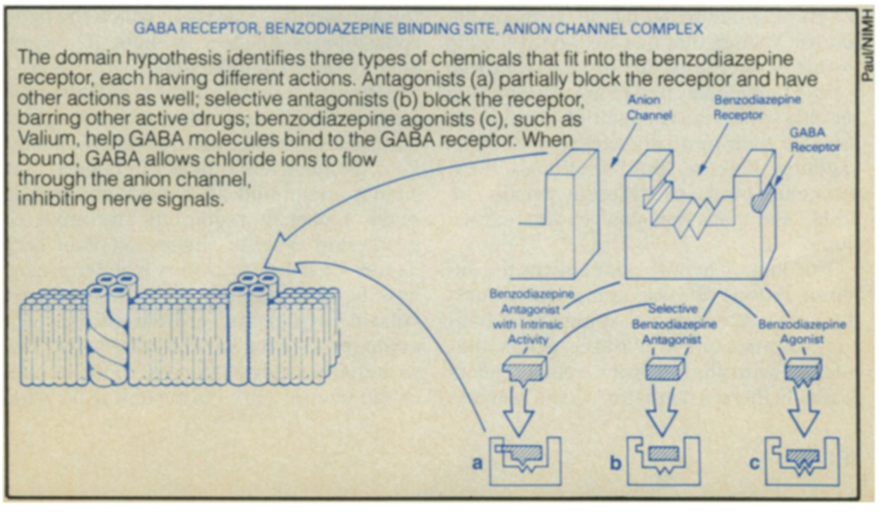
"If you think of it from an evolutionary standpoint," Skolnick says, "we probably wouldn't be here if our ancestors hadn't had a little bit of anxiety; they would have been eaten by saber-toothed tigers." Indeed, the flight-or-fight response exists for a purpose. It allows faster reaction and heightened sensibilities when trouble looms. But many scientists believe that this mechanism, designed to create anxiety in certain situations, sometimes goes awry. Evidence for this comes from the observation that, in some susceptible individuals, anxiety appears without a logical or apparent cause, putting people "on edge" in its milder forms and eliciting fear, confusion and neuroses in its more dramatic appearances. It can incapacitate and make life miserable for the suffering individual. From 2 to 5 percent of the general population suffers from anxiety so severe that they seek professional help. In view of this, it is not surprising that the benzodiazepines are the most widely prescribed drugs in therapeutic use today.

The mechanism by which benzodiazepines exert their behavioral and physiological changes was first revealed in 1977, when two independent research groups, led by Richard Squires of Rockland Research Institute in Orangeburg, N.Y. and Hermann Möhler of Hoffmann-La Roche in Basel, Switzerland, discovered a selective receptor for benzodiazepines in the brain. Later, NIMH researchers John F. Tallman, Erminio Costa and Alessandro Guidotti found that the benzodiazepine receptor is clustered on the nerve cell surface with a recognition site for gamma-aminobutyric acid (GABA) and a chloride ionophore—a channel that regulates the flux through the cell membrane of chloride ions, which control the nerve cells' firing rate. GABA is a naturally occurring chemical that transmits inhibitory messages, which quiet nerve cells within the brain. When it is released by one nerve cell into the synapse, or space between the cells, GABA acts on the next nerve cell membrane, increasing the membrane's permeability to chloride ions. This action decreases the cell's firing rate, thus decreasing brain activity.

Benzodiazepines have no effect when GABA is not present. And when both GABA and benzodiazepines are present, GABA functions even more effectively than when alone. More specifically, benzodiazepines increase the affinity of GABA for its receptor—the site on the membrane that recognizes GABA—rather than by altering the amount of GABA released or the number of receptors available. Most researchers believe that benzodiazepines act as a co-transmitter of GABA, enhancing its inhibitory actions. In those individuals with intense anxiety, this increased inhibition of nerve cell firing could induce more calm behavior.

No one knows yet how benzodiazepines exert this effect on GABA, although several research groups have differing hypotheses. Skolnick points out that "basically, we all agree that benzodiazepine receptors are coupled to a GABA receptor and [these receptors] are coupled to a chloride ionophore." He adds, however, that "the inner workings are not at all clear."

Yet Skolnick and Paul have quite a bit of evidence to back up their hypothesis that the benzodiazepine receptor is a complex recognition site with various domains for different compounds, which can turn the GABA system on or off. The first hint of this

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theory came through the use of a group of related compounds called beta-carbolines (BCCEs). Claus Braestrup of A/S Ferrosan Co. in Soeborg, Denmark, first isolated BCCE from the urine of psychiatric patients. Braestrup thought he had found an endogenous ligand, or natural Valium, for the benzodiazepine receptor (SN: 11/10/79, p. 325). Although the compound was later found to be artificially produced by the extraction process (SN: 2/9/80, p. 94), it demonstrated interesting effects when injected into rats. "[This compound] has been a very important tool for understanding how the receptor works," says Paul. "For example, in rats, it can antagonize [block] the effects of benzodiazepines through the receptors." And when BCCE is administered to primates, it causes an effect opposite to that of benzodiazepines—acute anxiety.

In one such experiment, Paul and colleagues injected BCCE into monkeys. Within minutes the animals exhibited behavioral and physiological signs of anxiety. Two human volunteers in Europe received doses of a closely related BCCE. Both subjects developed a strong inner tension described as a feeling of "impending doom," along with the physiological symptoms of acute anxiety. The symptoms were so intense that one volunteer had to be given a drug to reverse BCCE's effects.

If BCCE works through the benzodiazepine receptor, why is its effect a mirror image of benzodiazepine's? And why does a benzodiazepine derivative called Ro15-1788, which works through the same receptor, actually block the actions of both benzodiazepines and BCCE?

To explain the actions of these three drugs on the benzodiazepine receptor, Paul and Skolnick have developed a model called the "domain" hypothesis for the benzodiazepine receptor system. "We have very good evidence that the domain hypothesis is actually a real phenomenon," Skolnick says. The model allows for three types of molecules to fit in the benzodiazepine receptor: A benzodiazepine antagonist with intrinsic activity, such as BCCE; a selective benzodiazepine antagonist, such as Ro15-1788, which blocks the effects of both BCCE and benzodiazepines; and the benzodiazepine agonist. The agonist can be any benzodiazepine or a naturally occurring ligand, perhaps the natural Valium, that has anxiolytic activity —that is, decreases anxiety. Each of these types of molecules has an area of common binding within the recognition site that allows for their mutually exclusive binding, explains Skolnick. "But the tail part of the molecule binds to different areas," he adds, "and that's how you get different actions."

The domain hypothesis explains the different actions of these compounds, but it does not attempt to explain how benzodiazepines, or the other drugs that interact with the receptor, actually affect GABA. Is there a mediator? Costa believes one exists, and he and his colleagues have isolated a protein called GABA-modulin, which they believe may act as a coupler between benzodiazepines and GABA. Costa has found that GABA-modulin inhibits the high-affinity binding of GABA to its recognition site. When benzodiazepines are not present, GABA-modulin binds to the GABA receptor. He hypothesizes that when the benzodiazepine receptor sites are occupied by anxiolytic compounds, such as Valium, the configuration of GABA-modulin changes. The change — an addition of a phosphate group at one or more sites on the protein molecule — uncouples the GABA-modulin from the GABA binding site and allows GABA to bind to its receptor, which in turn opens up the chloride ion channel.

The evidence for this coupler activity, however, has only been reported recently, and has not been confirmed in other laboratories. Even so, Costa says that these data, soon to be published, demonstrate that benzodiazepines "facilitate their action by allowing GABA-modulin to be phosphorylated."

Now that scientists are beginning to grasp how the anxiety-generating mechanism works, another obvious question must be answered: Why is it there in the first place? "The high-affinity recognition site for benzodiazepine was not made because nature knew that benzodiazepines were coming along," Costa says. "It was made for something else." This reasoning has prompted other scientists to search for a naturally occurring anxiolytic — the natural Valium. Past efforts have led to the isolation of other endogenous compounds, such as the purines hypoxanthine and inosine (SN: 12/16/78, p. 424), but these are unlikely candidates because they bind so weakly to the receptor. Costa, in his search, has recently isolated a naturally occurring protein, called diazepam binding inhibitor (DBI), which binds strongly to the benzodiazepine receptor (SN: 6/18/83, p. 388). Like benzodiazepines, DBI displaces BCCE, but it has the same anxiogenic, or anxiety-producing, effects as BCCE. So instead of a natural anxiolytic, Costa believes he has found the exact opposite — a natural anxiogenic compound. "It makes sense," he says, "that the endogenous system in which the benzodiazepines operate is there to *create* anxiety, not to limit anxiety."

Although it is no longer a high-priority item, researchers have not given up on their search for the natural Valium. Costa, Paul, Skolnick and others still believe that such a compound does exist, and that it plays a part in regulating the anxiety-producing system. Higher on Paul and Skolnick's list of priorities is determining how benzodiazepines elicit the anticonvulsant actions and drowsiness that accompany the drug's anxiolytic effects. Paul says that roughly 30 percent of the nerves in the central nervous system work with GABA. However, GABA synapses are clustered in different areas of the brain. The general view is that different areas of the brain control different bodily functions. And recent evidence indicates that three different types of benzodiazepine receptors exist, each in a separate area of the brain. So the action of benzodiazepines on the cortex, for example, might yield an anticonvulsant effect, whereas benzodiazepines' action on the GABA receptors in the midbrain would cause drowsiness.

The relationships between the benzodiazepine receptor and the sedative and hypnotic properties of these compounds, however, are not firmly established. Paul and Skolnick have seen some interesting results using a BCCE derivative, 3HMC, which reverses the actions of flurazepam (a benzodiazepine widely prescribed for sleep disorders) in rats and induces a state of calm wakefulness. This observation, Paul says, suggests that benzodiazepines do function to regulate sleep. Also, 3HMC doesn't appear to cause seizures, whereas the other BCCEs do. So, Skolnick says, he and his colleagues could be on the verge of synthesizing compounds that have some, but not all, of the physiological effects of the benzodiazepines. He predicts that "within the next 10 years we will have anxiolytics that are not sedating."

Costa, on the other hand, has a much different goal. If he is correct, he says, and the naturally occurring co-transmitter is inhibitory of GABA, then he would speculate that certain sociopaths may have too much of this substance. "It is possible that certain criminals have a defect in this anxiogenic mechanism," he says. "They may not be able to generate anxiety or guilt." Costa's next research effort will be to produce a monoclonal antibody that can be used to measure DBI in spinal fluid.

The idea of a biochemical marker like DBI for mental illness or emotional upset is relatively new, and it raises, once again, the question of nature or nurture causing psychiatric disorders. Freud's followers believed that an individual's "dynamic unconscious" was the sole influence on his or her condition—whether it be anxiety or schizophrenia—and that the ideas that lay within the unconscious were derived from early childhood experience.

Within the field of psychiatry, a tug-of-war between these two schools of thought still exists. Although most scientists will agree that elements of both work to precipitate mental illness, the degree to which each plays a role is open to debate. Yet even Freud, with his lack of knowledge concerning the brain's biochemistry, visualized a day when scientists would build a bridge across the gap between the science of biochemistry and the practice of psychiatry. Costa's goal is to cross that bridge. "Psychiatry must become a science," says he, "and it can be a science only when its practitioners can measure things." □